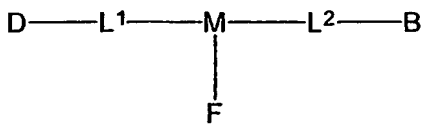
Claims

1. A compound comprising a cyanine dye or derivative thereof containing at least one target bonding group selected from a carboxylic acid thioester group or a group suitable for covalent reaction with a thioester, characterised in that said compound includes an affinity tag covalently bound thereto.

2. A compound according to claim 1 having the formula (I):



(I)

wherein:

D is a dye selected from a cyanine dye or a derivative thereof;

B is an affinity tag;

F comprises a target bonding group selected from a carboxylic acid thioester group and a 1,2-aminothiol group;

M is a group adapted for attaching to F; and

L^1 and L^2 each independently comprise a group containing from 1 – 40 linked atoms selected from carbon atoms which may optionally include one or more groups selected from –NR'–, –O–, –CH=CH–, –CO–NH– and phenylenyl groups, where R' is selected from hydrogen and C_1 – C_4 alkyl.

3. A compound according to claim 2 wherein each of L^1 and L^2 contains from 2 to 30 atoms.

4. A compound according to claim 2 wherein L^1 and L^2 are independently selected from the group:

$$-\{(CHR')_p-Q-(CHR')_r\}_s-$$

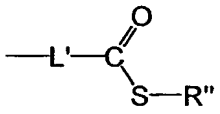
where Q is selected from: –CHR'–, –NR'–, –O–, –CH=CH–, –Ar– and –CO–NH–; R' is hydrogen or C_1 – C_4 alkyl, p is 0 – 5, r is 1 – 5 and s is 1 or 2.

5. A compound according to claim 4 wherein Q is selected from –CHR'–, –O– and –CO–NH–, where R' is hereinbefore defined.

6. A compound according to any of claims 1 to 5 wherein said affinity tag is selected from biotin and desthiobiotin.

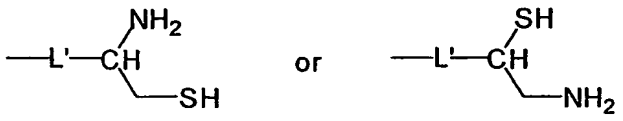
7. A compound according to any of claims 1 to 5 wherein said affinity tag is selected from his-tag, iminodiacetic acid and nitrilotriacetic acid.

8. A compound according to any of claims 1 to 7 wherein the target bonding group F is a carboxylic acid thioester of formula:



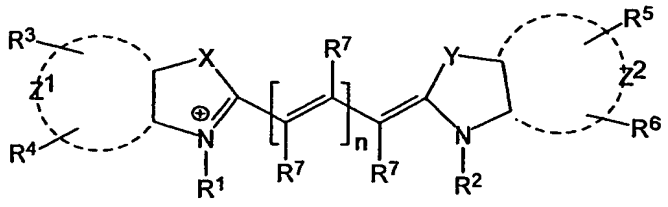
wherein L' is a bond or is a group containing from 1 – 30 linked atoms selected from carbon atoms and optionally one or more groups selected from –NH–, –O– and –CO–NH–; and R" is C_1 – C_4 alkyl, C_6 – C_{10} aryl, or C_7 – C_{15} aralkyl, which may be optionally substituted with sulphonate; or is the group $–(CH_2)_2–CONH_2$.

9. A compound according to any of claims 1 to 7 wherein the target bonding group F is a 1,2-aminothiol group of formula:



wherein L' is hereinbefore defined.

10. A compound according to any of claims 1 to 9 wherein the compound has the formula (II):



(II)

wherein:

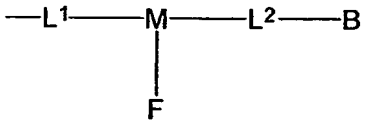
groups R^3 and R^4 are attached to the Z^1 ring structure and groups R^5 and R^6 are attached to the Z^2 ring structure;

n is an integer from 1 to 3;

Z^1 and Z^2 independently represent the atoms necessary to complete one ring or two fused ring aromatic or heteroaromatic systems, each ring having five or six atoms selected from carbon atoms and optionally no more than two atoms selected from oxygen, nitrogen and sulphur;

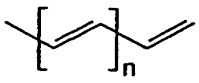
X and Y are the same or different and are selected from: $>CR^8R^9$, oxygen, sulphur, –CH=CH–, >N–W wherein N is nitrogen and W is selected from hydrogen and the group R^{10};

at least one of groups R^1, R^2, R^3, R^4, R^5, R^6, R^8, R^9 and R^{10} is the group:



where B, F, M, L^1 and L^2 are hereinbefore defined;

groups R^7 are independently selected from hydrogen and $C_1 - C_4$ alkyl which may be unsubstituted or substituted with aryl, or two or more of R^7 together with the group:



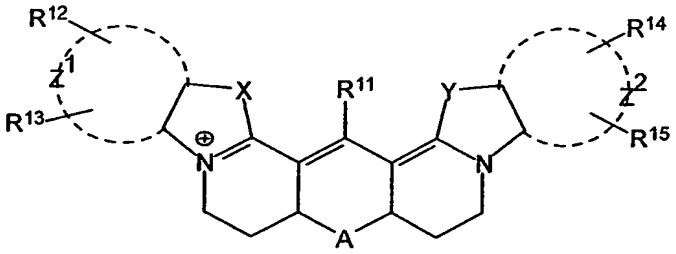
form a hydrocarbon ring system substituted with R^7 and which may optionally contain a heteroatom selected from –O–, –S– or >NR^7, wherein R^7 and n are hereinbefore defined;

remaining groups R^3, R^4, R^5 and R^6 are independently selected from the group consisting of hydrogen, halogen, amide, cyano, nitro, mono- or di-C_1 – C_6 alkyl-substituted amino, carbonyl, carboxyl, C_1 – C_6 alkyl, C_1 – C_6 alkoxy, aryl, heteroaryl, aralkyl and the group –$(CH_2)_m$–Y where Y is selected from sulphonate, sulphate, phosphonate, phosphate and quaternary ammonium and m is zero or an integer from 1 to 6;

remaining groups R^8, R^9 and R^{10} are independently C_1 – C_6 alkyl; and

remaining groups R^1 and R^2 are independently selected from hydrogen, C_1 – C_{10} alkyl, the group –$(CH_2)_m$–Y wherein Y and m are hereinbefore defined, and benzyl which may be unsubstituted or substituted by up to two nitro groups.

11. A compound according to any of claims 1 to 9 wherein the compound has the formula (III):



(III)

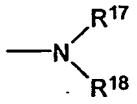
wherein

groups R^{12}, R^{13}, R^{14} and R^{15} are attached to the rings containing X and Y or, optionally are attached to atoms of the Z^1 and Z^2 ring structures;

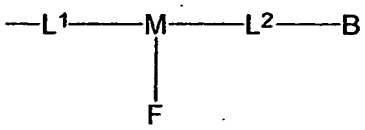
Z^1 and Z^2 independently represent the atoms necessary to complete one ring or two fused ring aromatic or heteroaromatic systems, each ring having five or six atoms selected from carbon atoms and optionally no more than two atoms selected from oxygen, nitrogen and sulphur;

X and Y are the same or different and are selected from: $>CR^8R^9$, oxygen, sulphur, –CH=CH–, >N–W wherein N is nitrogen and W is selected from hydrogen and the group R^{10};

A is selected from O and NR^{16} where R^{16} is the substituted amino radical:



at least one of groups R^8, R^9, R^{10} R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{17} and R^{18} is the group:



where B, F, M, L^1 and L^2 are hereinbefore defined;

remaining groups R^{11}, R^{12}, R^{13}, R^{14} and R^{15} are independently selected from the group consisting of hydrogen, halogen, amide, cyano, nitro, mono- or di-$C_1 - C_6$ alkyl-substituted amino, carbonyl, carboxyl, $C_1 - C_6$ alkyl, $C_1 - C_6$ alkoxy, aryl, heteroaryl, aralkyl and the group $-(CH_2)_m-Y$ where Y is selected from sulphonate, sulphate, phosphonate, phosphate and quaternary ammonium and m is zero or an integer from 1 to 6;

remaining groups R^8, R^9 and R^{10} are independently $C_1 - C_6$ alkyl;

remaining group R^{17} is selected from hydrogen, $C_1 - C_4$ alkyl and aryl; and

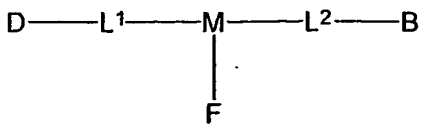
remaining group R^{18} is selected from $C_1 - C_6$ alkyl, aryl, heteroaryl, an acyl radical having from 2-7 carbon atoms, and a thiocarbamoyl radical.

12. A compound according to claim 10 or claim 11 wherein Z^1 and Z^2 are selected independently from the group consisting of phenyl, pyridinyl, naphthyl, quinolinyl and indolyl moieties.

13. A compound according to claim 10 or claim 11 wherein Z^1 and Z^2 are selected from phenyl and naphthyl moieties.

14. A method for labelling a protein of interest wherein said protein contains or is derivatised to contain an N-terminal cysteine, the method comprising:

i) adding to a liquid containing said protein a compound of formula (I):



(I)

wherein:

D is a dye selected from a cyanine dye or a derivative thereof;

B is a bioaffinity tag;

F comprises a target bonding group selected from a carboxylic acid thioester group and a 1,2-aminothiol group;

M is a group adapted for attaching to F; and

L^1 and L^2 each independently comprise a group containing from 1 – 40 linked atoms selected from carbon atoms which may optionally include one or more groups selected from –NR'–, –O–, –CH=CH–, –CO–NH– and phenylenyl groups, where R' is selected from hydrogen and C_1 – C_4 alkyl; and

ii) incubating said compound with said protein under conditions suitable for labelling said protein.

15. A compound according to claim 14 wherein each of L^1 and L^2 contains from 2 to 30 atoms.

16. A method according to claim 14 wherein L^1 and L^2 are independently selected from the group:

$$-\{(CHR')_p-Q-(CHR')_r\}_s-$$

where Q is selected from: –CHR'–, –NR'–, –O–, –CH=CH–, –Ar– and –CO–NH–; R' is hydrogen or C_1 – C_4 alkyl, p is 0 – 5, r is 1 – 5 and s is 1 or 2.

17. A method according to claim 16 wherein Q is selected from –CHR'–, –O– and –CO–NH–, where R' is hereinbefore defined.

18. A method according to any of claims 14 to 17 further comprising separating and/or purifying the dye-labelled protein of interest by affinity chromatography.

19. A method according to any of claims 14 to 18 wherein said protein of interest is selected from antibody, antigen, protein, peptide, microbial materials, cells and cell membranes.